2014 IMPAKT BREAST CANCER CONFERENCE

8-10 May, 2014

Brussels, Belgium

Table of Contents

Summary

The 6th IMPAKT (IMProvingcAre and Knowledge through Translational research) Breast Cancer Conference, held 8-10 May 2014 in Brussels, Belgium brought together leading investigators and experts in the field of translational research in breast cancer. Organised annually by the European Society for Medical Oncology (ESMO) and the Breast International Group (BIG), this year's subject "Anticipating the future of personalised medicine in breast cancer" brought together a unique community of professionals to facilitate the advancement of a personalised approach to breast cancer management and the implementation of new discoveries into clinical practise. The conference provided an excellent forum for the most recent translational research findings in breast cancer and gave insights into how these data may alter patient care.

IMPAKT is organised in collaboration with a multidisciplinary alliance of European breast cancer organisations and patient groups - referred to as partners – including the Foundation St. Gallen Oncology Conferences and the EBC Council.

IMPAKT is designed for breast cancer researchers and clinicians who have a specific interest in translational research, new agents, molecular and functional diagnostics, biomarkers and cutting-edge research applications in the clinical setting.

The scope of this report is to present the scientific highlights of the IMPAKT 2014 Conference.

Introduction

More than 530 participants came from 56 countries to present and learn about the latest technological advances, research findings and emerging clinical care strategies in the field of breast cancer. Importantly, the conference built a bridge between new data (right from the level of basic research) and clinical practise. Laboratory discoveries were translated and tested in clinical practise environments in clinical trials and hypotheses refined with investigators, allowing them to build upon this knowledge and move forward in their further investigations.

Among the major themes of the conference were the use of patient-derived xenografts to dissect the mechanisms of resistance to systemic therapies; liquid biopsy, monitoring tumour-derived cell-free DNA, miRNA, circulating tumour cells (CTCs) and exosomes in patients with breast cancer; pathways for drug discovery and development from next generation genomics to next generation therapies; mathematical modelling to map the subway of biological networks;

and metastatic dormancy, in particular investigating early dissemination and tumour cell quiescence.

A specific pre-IMPAKT training course was aimed at providing early-career professionals with the 'must know' fundamentals of research and novel technologies used in the field of breast cancer. The course was attended by 78 young doctors and scientists. The programme was based on the ESMO/BIG Curriculum in Translational Research in Breast Cancer. Both, ESMO and BIG recognised a gap in the formal educational criteria for medical professionals involved in translational research activities in breast cancer. Representatives from both societies worked together to develop a document that will support and better coordinate the translational research training of medical personnel by emphasising the basic subjects to be considered within the standardised curriculum's outline.

The oral and poster presentations provided information about advances made in breast cancer research on the molecular level and created an understanding of the way in which changes on this level may determine response to treatments used in clinical practise.

The conference was designed to encourage networking and provided opportunities for attendees to meet experts in the breast cancer field, share information and form new collaborations. Participants left the conference with not only a high-quality overview of current translational research and personalised medicine achievements, but also new inspiration and renewed focus in their endeavour to provide the best medical care available to their patients with breast cancer.

The programme of 2014 IMPAKT Breast Cancer Conference began with reports on patient-derived tumour xenografts.

Patient-Derived Xenografts

Xenograft models of solid tumours

Dr Geoff Lindeman of The Walter and Eliza Hall Institute & Royal Melbourne Hospital reviewed the advantages and limitations of current xenograft models for the study of tumour biology and potential therapeutic agents: cell lines (selected through multiple passages, acquisition of multiple mutations), animal tumour models (genetic modification/mutagenesis resulting in tumour predisposition; differences between species), cell line derived xenografts (selected through multiple passages, acquisition of multiple mutations; do not recapitulate tumour heterogeneity; rarely form metastases), and patient-derived xenografts (PDX) models (often recapitulate tumour heterogeneity and behaviour; share genomic features with the primary tumour; may form metastases).

Tumour latency is generally measured over months, making 'real-time evaluation' for patients a challenge. However, only a proportion of primary breast tumours engraft. 'Take rate' is likely to depend on a variety of factors: immunodeficient model, source (primary vs. metastasis), site (orthotopic/cleared mammary fat pad, subcutaneous fat), matrigel and stromal cells, oestradiol supplementation is often required.

Opportunities of PDX models include:

(1) Recapitulates tumour heterogeneity
- A preferred model for *in vivo* cancer stem cell studies
- Clonal representation is maintained on transplantation
- Luminal xenografts retain hormone receptor heterogeneity and endocrine responsiveness

(2) Amenable to discovery research
- Early passage (treatment-naive) PDX models may select for the subset of cells prone to metastasis
- Enable genomic studies that identify driver mutations (eg. *ESR1* variants)
- Study metastasis
- Lentiviral transduction, *in vivo* imaging and cell tracing, 'humanisation'

(3) Renewable source of tumour
- Tumour sphere assays, dissociated tumour cultures

(4) Offer pre-clinical models for evaluation novel therapies (response/resistance)
- Xenograft models of solid tumours are powerful new research tool to study tumour behaviour, reveal the potential utility of novel therapies, and evaluate personalised therapy based on distinct genomic features of the tumour.

Xenograft models: Problems, pitfalls and future developments

Dr Angelo Di Leo of the Hospital of Prato, Istituto Toscano Tumori, started his talk by summarising the clinical research context in 2014:

a) Neo-adjuvant and "window of opportunity" studies facilitate *in vivo* understanding of the pharmacodynamics of new agents;
b) Access to biological samples is becoming common in the context of clinical studies (tumour tissue, circulating tumour cells - CTC, circulating biomarkers);
c) Increasing knowledge is becoming available on drugs mechanism of action and tumour biology.

He reviewed studies in which patient-derived breast cancer xenografts were generated. In total twelve published studies were identified, however the bottom-line message from the review of these studies is that while there good evidence that PDX are consistent with the tumour of origin in terms of morphology, genomics, transcriptomics and proteomics, there is very limited evidence that response to anti-cancer agents in the PDX reflects response to the same agent in the patient of origin.

Breast cancer studies comparing response to a given agent in the PDX and in the matched tumour of origin was studied in 2 out of 12 studies. There are a number of issues limiting our ability to draw any conclusions: limited experience (only 20 cases PDX/patient reported overall), limited number of agents (essentially doxorubicin or docetaxel), response assessment in patients implies evaluation of multiple metastatic sites (only the fat pad site in the PDX), treatment duration ≤ 9 weeks with implications on no data on late responses, and no data on acquired resistance. In addition, there is no information on the quality of the observed response in term of duration, and disease stabilisation.

Additional issues to consider include the immune system of the host - these are severely immuno-compromised with potential risk of under-estimating treatment activity with the PDX model, particularly in cases where immuno-competence is relevant; and the engraftment rate - in luminal tumours the engraftment rate is low (2.5% vs. 24.7% in non-luminal cancers) - the engraftment rate can potentially be improved by stimulating tumour growth at the fat pad site (by oestrogens, matrigel, human mesenchymal stem cells, …). In addition, there is potential selection bias is that only highly proliferating tumours will be engrafted successfully. Last, but not least, it is questionable if PDX can reproduce the complex intratumour heterogeneity of their tumours of origin, if the lack of the human stromal component in the PDX model alters the biology and the behaviour of the engrafted tumours, and if results observed with the PDX model reproducible across different laboratories?

Di Leo concluded that PDX is a reliable pre-clinical model to investigate drug activity in breast cancer. However, only preliminary experience is available, particularly with regard to the comparison in terms of response to anti-cancer agents between PDX and the matched tumour of origin. It is potentially an inadequate model to assess the activity of anticancer agents requiring a functional immune-system and/or interacting with the stroma component, and still disappointing for luminal tumours.

Breast cancer Avatars: Patient-derived xenografts as a platform for drug development

Dr Michael Lewis of the Baylor College of Medicine started his talk with human clinical trial limitations, the inability to have untreated patients as controls, predict which patients will respond a priori, treat same patient with multiple therapies, and evaluate scheduling efficiently. Number of treatment arms is limited by the number of patients available, ethical considerations, and financial considerations.

Dr Lewis raised several questions. Can xenograft-bearing mice serve as useful avatars and if so, can resistance mechanisms be discovered more efficiently, can we develop predictors of differential treatment response, can we change the way in which drug evaluation is done and can we reduce the cost of drug development?

The advantages of animal clinical trials are the ability to transplant dozens to hundreds of tumours, , randomise each tumour to multiple treatment arms, (as many as it is possible to manage), compare outcomes across treatment arms, correlate outcome with patient of origin and xenograft-based molecular data in term of single agent response prediction, drug combinations and sequencing, resistance mechanism discovery, superiority (”go/no go” decision making in drug development) and possible translation to clinical decision making.

Dr Lewis said that breast cancer PDX resources are available in the Curie Institute (France), Baylor College of Medicine (USA), University of Utah (USA), Washington University (USA), University of Colorado (USA), Walter and Eliza Hall Medical Research Institute (Australia). This list highlights a lack of breast cancer PDX resources in Europe.

A renewable tissue resource of phenotypically stable biologically and ethnically diverse, patient-derived human breast cancer xenograft models represent all clinically-defined subtypes,

histologically nearly identical to tumour of origin, cluster molecularly with human tumours, phenotypically stable at the genomic, transcriptomic, and proteomic levels, and treatment responses match tumour of origin.

When planning animal clinical trials you should consider how many xenograft models are required, how many animals per treatment arm, are all chemotherapy agents really identical, can differential response predictors be identified, and what is the cost to conduct an animal clinical trial.

In term of cost comparison, a phase I human trial with two arms and 30 patients per arm (60 in total) costs 1.5-2.5M USD, but provides little or no efficacy data, no correlative biomarkers are discoverable, and no predictive analysis is possible. By contrast, a phase I animal study with two arms and 30 PDX/arm (30 in total) costs 50-60K USD. Quantitative efficacy data are possible, correlative biomarkers discoverable, and predictive analysis is possible.

Dr Lewis concluded that representative models exist; they accurately reflect patient biology, accurately reflect patient treatment response, differential treatment response prediction should be possible to inform phase II studies, and they are cost effective relative to human phase I studies.

Animal models and resistance to HER2 targeted therapy

Dr Kent Osborne of the Baylor College of Medicine spoke about oncogene addiction and treatment when a cell is driven by a single powerful driver pathway. Other redundant survival pathways become inactive because they are not needed, but can be reactivated if the driver is blocked. Potent inhibition of the driver pathway should result in cell death.

Optimal targeted therapy should identify key pathway(s) and the driver; block this pathway completely; anticipate escape (resistance) mechanisms and block them; and could be used within combination therapy.

HER2-positive breast cancer is the ideal tumour to apply these principles.

The positives of using human tumour xenografts in mice are that they are relatively cheap; large experiments are possible; there are many cell lines (oestrogen receptor (ER)-positive, HER2-positive, triple negative); results are reproducible; work well with targeted therapies; tissue for molecular studies is available. Disadvantages are immune deficient mice, mouse stroma, and tumour growth kinetics.

He concluded that no model is perfect but human cell lines, xenografts, and PDXs can be helpful in predicting benefit in patients with HER2-positive breast cancer. These models can also be useful in understanding mechanisms for resistance. These models should be very useful in identifying the best drug combinations of the many choices to test in patients.

Genomic Research

Genome-forward trials and design challenges

Dr Lisa Carey of the University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, said that breast cancer treatment is informed by old-fashioned trials that were large, rigorous, and provided level I evidence-based medicine. She asked how, in light of modern technology, we can transform further. Challenges to meaningful clinical trials in the 'omic' era are:

1. Term "breast cancer" no longer exists, it is now a fragmented group of biologically-distinct entities.
2. Patients have much better outcome across the spectrum of the disease (good for patients, but bad for event rates). This poses a question how to develop drugs and treatment approaches if cancer is a group of orphan diseases.

Historical transformative trials (e.g. ATAC, CALGB974, HERA, …) were large, clearly defined, rigorous, with often modest effects, but these would be nearly impossible now because they were too big, too unselected, and there are too many competing good ideas.

Trials in the 21st century should be small, fast (collaboration is key), rational, careful (with regards to marker development and trial design).

Facts in favour of genome-forward trials include that they are biologically rational, increasingly feasible, and their enriched population can set success threshold high; conversely they carry a screening burden, they're expensive, and there are challenges when selecting the target: is this really a driver, is the assay valid, does the drug hit the target. In addition predicting targetability is not easy.

Trial designs popular now are:

- Umbrella (assign defined cancer type by molecular aberration)
- Basket (assign by molecular aberration regardless of tumour type)
- Neoadjuvant (smaller, faster, allow tissue studies)
- Window (study biology and resistance)
- Adaptive (can be used with several designs, smaller, nimble).

Umbrella trials (e.g. SAFIR01, AURORA) consider genotyping all patients of a particular disease and allocating patients to particular drugs based upon profiling results. They are appealing and rational, however they often depend on unproven drug(s), and unproven assays, selecting appropriate endpoints can be more difficult.

Basket studies (e.g. NCI-MATCH, Novartis Signature Trials) have same pros as umbrella studies: they are appealing and rational; however, cons are: lineage specificity, biomarker reliability/accuracy, and same endpoint problems.

Window of opportunity trials provide proof of principle, biomarker discovery (study biology and resistance); however they can't use really new agents (safety issues), hard to test combinations. They contribute to scientific knowledge and therapeutic hypotheses, but not to clinical care.

Neoadjuvant trials have an additional advantage: they allow you to 'pick a winner', tissue samples available pre-treatment and at surgery, pathologic complete response (pCR) is a good surrogate endpoint in some tumour types (with a possible FDA registration option), disease-free survival/overall survival can be collected in same patients (may be underpowered); cons: pCR is only validated endpoint in some breast cancers (irrelevant in many ER-positive tumours), a quantitative relationship between pCR disease-free survival/overall survival is not established, it is unknown if macrometastasis is equal to micrometastasis, drugs must be well known. They can contribute to clinical care, represent excellent way to get clinical plus biologic information. However, the only trials with significant impact on outcome have been in the HER2+ setting).

Residual disease trials have different pros: tissue available, resistant tumours, high risk population; however, the cons are: they are large trials, cannot assess response (event = relapse), need to be randomised.

Adaptive trials allow you to 'pick a winner', can be adapted to a drug or biomarker, conserve resources, are faster, but not necessarily smaller; cons are: potential lack of reliability of interim estimates, higher error risk, complicated.

Precision medicine is coming…but how precise? There are real challenges to trial design in the genomic era re power, effect size, and adequate diagnostic assays. We will need to rely on smaller, less stringent clinical trials and pharmacodynamic studies. Modern challenges include the increasing number of known genetic aberrations (hundreds across the breast cancer spectrum – many of which affect small populations e.g. HER2 activating mutations, androgen receptor (AR)); uncertainty about identifying targetability (gene/RNA/protein); explosion of biologically-directed agents (single agent, combinations, circumventing resistance); tumour evolution and tumour microenvironment.

Whole genome sequencing and cancer therapy: what is too much?

Dr Jorge Reis-Filho, attending pathologist in the Department of Pathology and affiliate member in the Human Oncology and Pathogenesis Program of the Memorial Sloan Kettering Cancer Center, started his talk with the USA National Academy of Sciences 2011 definition of precision medicine: "The use of genomic, epigenomic exposure, and other data to define individual patterns of disease, potentially leading to better individual treatment".

Dr Filho said that rare driver genes can be missed, for example ESR1 mutations are present in 0.6% of luminal tumours, and HER2 mutations in approximately 1.5% of breast cancers. Factors to consider are that not all tumours have identifiable driver mutations, not all drivers have been identified, there is an incomplete characterisation of drivers, drivers of metastatic disease, drivers of resistance to specific agents, and we are at beginning of understanding of epistatic interactions (e.g. mutation A plus mutation B results in a different phenotype).

There are several approaches for massive parallel sequencing and therapy decision making, including whole genome sequencing, targeted capture sequencing, whole exome sequencing, and whole exome sequencing plus RNA sequencing.

Whole genome sequencing considers all somatic genetic aberrations. There is some uncertainty for single nucleotide variants (SNVs) and it is still problematic for indels; fusion gene

identification is not a trivial exercise: validation with orthogonal methods is required which is still expensive; and requires a lot of computer power and army of bioinformaticians.

Targeted capture sequencing is an excellent option if we consider that breast cancers are driven by a limited constellation of known driver mutations, fusion genes and copy number aberrations; and if we can target the functional impact of each mutation.

Whole exome plus RNA sequencing is an excellent approach, but...what do we do with the incidental findings?

As take home messages, Dr Filho stated: sequencing for therapy decision making is dependent on the use intended; for enrolment in clinical trials targeted capture sequencing (including selected intronic regions); for patients in the metastatic setting after multiple lines of therapy targeted capture sequencing (including selected intronic regions) and exome plus RNA sequencing could be used; whole genome sequencing is unjustified at present.

Overcoming operational challenges of personalised cancer therapy

Dr Fraser Symmans of the Department of Pathology, MD Anderson Cancer Center said that in the SAFIR01 study 423 patients were consented, but this represents only a minority of new metastatic patients. There were 195 “targetable” mutations; 23 (5%) patients had standard of care altered due to this trial, of these 13 (3%) had clinical benefit from their therapy. Therefore while this trial is important for research it didn’t change standard of care for 95% of patients. No evaluation of the psychological/QoL consequences of this unactionable extra knowledge was foreseen.

Further in his talk, Dr Symmans spoke about clinical biomarker testing: does it have a real clinical utility or irrational exuberance? Multidisciplinary decision team effort is needed, as well as to distinguish between clinical validity based on level of evidence and “actionable” interest based on hypothesis.

Clinical next-generation sequencing (NGS) requires high coverage depth: the trade-off in generating so many parallel sequences using PCR/DNA polymerase is loss of accuracy; NGS platforms have approximately 10-fold higher error rates (1 in 1000 bases) versus Sanger sequencing (1 in 10,000 bases); for clinical accuracy, each template requires 100’s of sequence reads to account for sequencing errors, non-neoplastic DNA “contamination”, and artefacts from formalin.

According to Clinical Laboratory Improvement Amendments (CLIA), NGS of solid tumours requires high coverage: multiple (~500x) reads of the same sequence to gain confidence in the result. It is critical when the ratio of neoplastic to non-neoplastic cells is low, as it allows the signal to be sifted from the noise, examination of reads in both directions to rule out artefacts, and to confirm or rule out sequence variant using an additional method (e.g. Sanger).

The adequacy of a sample for histologic diagnosis vs. adequacy for biomarker testing is a very difficult issue; it is not unusual to make a diagnosis of cancer from only a few cells, these same cases might be unsuitable for molecular testing. The focus should be on tissue qualification in pathology, and engagement of interventional radiologists and the cytologists.

Clinical testing for personalised cancer therapy requires active participation from a truly multidisciplinary decision , seeking clinical validity and clinical utility before implementation as standard of care (otherwise, it is reasonable to perform this within a clinical trial), only performed in an accredited diagnostic laboratory (procedures and requirements are different from research), quality of the sample is critical to success (ideally collect samples with the best quality molecules, otherwise, sample qualification is essential), it is needed to demonstrate feasibility with limited samples.

Bioethics in Genomic Research

Whole genome research: Ethical, legal and social implications

Dr Harriet Teare of the HeLEX Centre, University of Oxford discussed how research is changing and illustrated this using the human genome project, which represents the first ‘big science’ project in genomics. It was not hypothesis-led but contributed to establishing an infrastructure, change from competitive to co-operative approach, co-ordination of groups around the world, interdisciplinary teams with specific skills, and open access to data.

It is difficult to keep the identity of participants anonymous when data is shared in open access web repositories, but not sharing goes against current funders’ policies and scientific need for large sample sizes.

As examples of the issues of preserving privacy, she referenced an article where individuals in genome-wide association study (GWAS) were identified using only summary statistics, and a separate re-identification of male participants using single nucleotide polymorphisms (SNPs) on Y chromosome, linked to data found in publicly available datasets on Internet.

Furthermore she touched on a problem of incidental findings, the findings that are not part of the original research aims, but have clinical significance. She questioned a comprehensive nature of whole genome sequencing and what should we feedback in terms of individual level findings, who decides and who should feedback findings to whom, how should we protect the right ‘not to know’, what is the responsibility of the researcher to look, feedback, and beyond the original project scope?

Regarding consent and withdrawal of consent, she spoke about proposed EU data protection regulation and actions to remove the current exemptions applied for medical research, namely the ability to keep data indefinitely, or a use samples or data for secondary research purposes without explicit consent. She also covered the tightened requirements for consent, specifically that it should be freely given, specific, informed and explicit. It is questionable whether broad consent is lawful.

Consent forms in genomics: Assessing the privacy risks of data sharing

Dr Katherine Nathanson of the Abramson Cancer Center, University of Pennsylvania School of Medicine suggested that consent and data privacy issues are related to germline sequence data, and not tumour sequence (most tumour samples will contain both material). There are multiple approaches to somatic sequencing, which are non-equivalent in their use of germline data and each of these requires a different approach to consenting. An individual physician's decision about consenting tends to depend upon time, support and concerns about legal liability.

Sources of germline findings in tumour mutation profiling can be indirect (germline DNA sequence reflected in DNA of tumour) and direct (germline DNA sequence determined for comparison to tumour sequence).

Defining the somatic variants to evaluate for potential germline status, is important to ascertain that the gene mutation is consistent with phenotype (it is important to go back to family history), known founder mutations (e.g. Ashkenazi Jewish BRCA1/2 mutations), biallelic mutations in the tumour, functionally significant (not variants of uncertain significance), allelic ratio in tumour.

The consent required depends on the sequencing plan. Where tumour sequencing alone will be performed, generally no consent is required; where tumour sequencing, subtracting out the germline, is performed either a short consent is required if only germline mutations found during quality control processes would be reported back, or a full consent is required if there is the option to provide germline data back as part of a research protocol – in this case the consent should include the extent of the returned germline results e.g. just cancer susceptibility genes or other medically actionable genes as well.

The incidentalome is the mutations and gene variants unrelated to the phenotype being studied. Focus is on medically actionable genes (e.g. LDLR mutation - familial hypercholesterolemia in patient sequenced for a brain tumour, autosomal recessive carrier status, and pharmacogenetic metabolism variants).

Dr Nathanson referred to the American College of Medical Genetics and Genomics (ACMG) recommendations for reporting of incidental findings in clinical exome and genome sequencing. However, longstanding issues in genetics continue to be discussed in the context of tumour/normal sequencing. She questioned what are our 'duty to seek' and 'duty to warn'?

Somatic mutation sequencing in any institution needs to be developed in concert with attention paid to how germline data will be dealt with and returned. Each institution may deal with these issues differently. There are different approaches particularly between whether somatic sequencing comes from an oncology-based or genetics-based laboratory. It is important to consider questions of consent and data privacy ahead of time and have a plan.

Circulating Tumour Cells: Isolation, Enrichment, and Clinical Value

Dr Michail Ignatiadis of the Institute Jules Bordet said that there is now level I evidence that CTC detection using CellSearch is an adverse prognostic factor in metastatic breast cancer and ongoing clinical trials are testing its clinical utility. The ongoing Treat CTC trial is testing CTC elimination as an early signal of trastuzumab activity in HER2-negative early breast cancer. The role of more sensitive CTC detection technologies or ctDNA for monitoring minimal residual disease in the early breast cancer setting should, in his opinion, be further explored. Plasma ctDNA should be prospectively tested as a tool for treatment selection and monitoring in clinical trials of patients with metastatic breast cancer. Technological advances have allowed CTC analysis as a 'liquid biopsy' to study tumour evolution. CTC analyses offer a unique window of opportunity to assess treatment resistance at the cellular level.

Targeting the CDK4/6 Pathway

Dr Luca Malorni of the Hospital of Prato introduced the CDK4/6 pathway and provided a background of our pre-clinical knowledge of CDK4/6 in breast cancer subtypes, namely luminals, HER2, and triple negative tumours. He said that mechanisms of bypass of HER2 targeted agents are complex (aberrant cellular proliferation in the presence of agents, common deregulated signalling that feeds into CDK4/6) and that CDK4/6 inhibition has shown activity inHER2 positive models. Markers of resistance (p16 and RB) are identified in clinical specimens. CDK4/6 inhibitors cooperate with HER2 targeted agents. Furthermore, he summarised available clinical data with CDK4/6 inhibitors: palbociclib, bemaciclib, and LEE001, as well as provided a review of currently ongoing clinical trials.

Selected Highlights from the Abstract-Related Sessions

Molecular subtype of a breast cancer relapse influences patient post-relapse survival: Translational aspects of Swedish multicentre, randomised phase III TEX trial

Translational results from the randomised phase III TEX trial show that breast cancer relapse characteristics display aggressive features with an over-representation of ER-negative, HER2-positive and highly proliferative tumours. The molecular subtype of breast cancer metastases significantly influences post-relapse survival, according to the presentation of Dr Nicholas Tobin of the Oncology-Pathology Department, Karolinska Institute. The study was presented at the Best abstracts session.

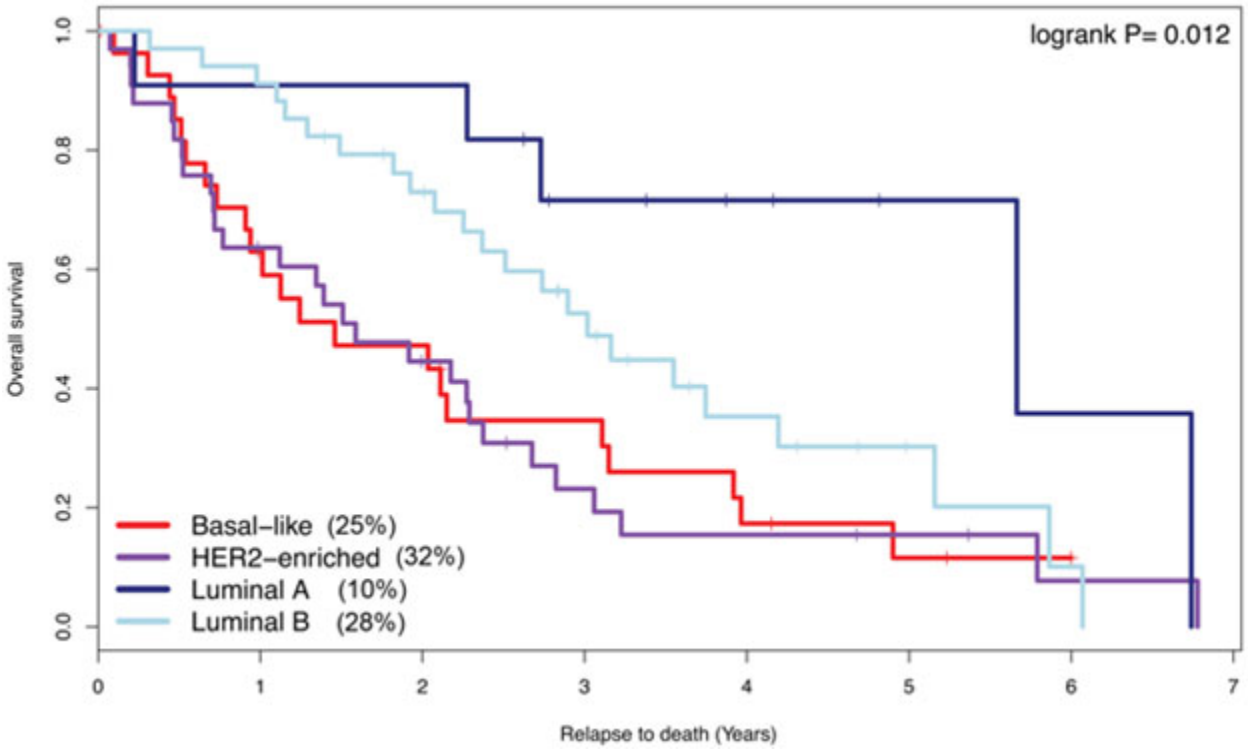
Breast cancer researchers have previously demonstrated the propensity of standard breast cancer markers to alter their expression throughout tumour progression with a subsequent impact on patient survival. Investigation of tumour characteristics at relapse has the potential to improve patient management and survival. The aim of the translational part of the TEX study was to better understand the biology of breast cancer metastases and how they can influence patient post-relapse survival. The researchers applied molecular markers with previously

demonstrated biological relevance and prognostic significance in the primary tumour setting to the metastatic samples from the TEX trial.

The translational part of the trial included 120 relapse biopsies from 111 patients, yielding sufficient tumour RNA for gene expression profiling. Each gene expression array was individually background corrected and normalised using robust multichip averaging. The gene expression modules and the PAM50 intrinsic subtypes were assessed.

Metastatic sites included breast in 14% of cases, liver in 23%, lung/pleura in 2%, lymph node in 36%, skeleton in 5%, skin in 19% and other sites in 1%.

Gene modules showed an over-representation of aggressive relapse tumour characteristics including low ER signalling, as well as high proliferation, HER2 and angiogenic signalling. In particular, a low ESR1 module score was associated with poor post-relapse survival. A low CASP3 module score was associated with poor post-relapse survival.



Caption: PAM50 subtypes are associated with poor post-relapse survival. © Nick Tobin

The PAM50 intrinsic breast cancer subtypes revealed that 25% of relapses were basal, 32% HER2, 10% luminal A, 28% luminal B, and 5% normal-like. Importantly, intrinsic subtype at relapse was significantly associated with post-relapse survival ($p = 0.012$).

Contrasting two consecutive relapse biopsies from the same patient, using hierarchical clustering of gene module genes, the researchers noted that 2 out of 7 patients exhibited different gene expression patterns, suggesting intra-tumoural heterogeneity in these relapses.

The Swedish investigators concluded that their findings indicate that the molecular subtype of a breast cancer relapse significantly influences post-relapse survival. Molecular investigations at breast cancer relapse may provide prognostically relevant information with the potential to improve patient management and post-relapse survival.

The authors have declared no conflicts of interest.

Prof. Peter Dubsky, who discussed the results, said that the findings represent an elegant proof of the principle that individual types of breast cancer change during disease progression. The data clearly adds detailed biology to what we know from immunohistochemistry. However, the technique used is currently of uncertain clinical validity. It is unlikely that it could detect more “actionable changes” than immunohistochemistry and it is with a little potential to find new actionable targets.

Adoption of multi-gene assays in hormone receptor-positive, HER2-negative breast cancer: Results of European survey on genomics application in clinical practise

There is substantial heterogeneity in the adoption of multi-gene assays in Europe. In a survey among physicians with at least 5 years of experience in treatment of breast cancer, a majority of respondents indicated that they would use multi-gene assays in clinical practise in a subset of patients with hormone receptor (HR)-positive, HER2-negative disease. The main perceived barriers for usage are reimbursement, price, and lack of availability, according to the survey’s results presented in a poster session on Genomics and proteomic analysis of breast cancer by Dr Matti Aapro of the Multidisciplinary Oncology Institute in Geneva.

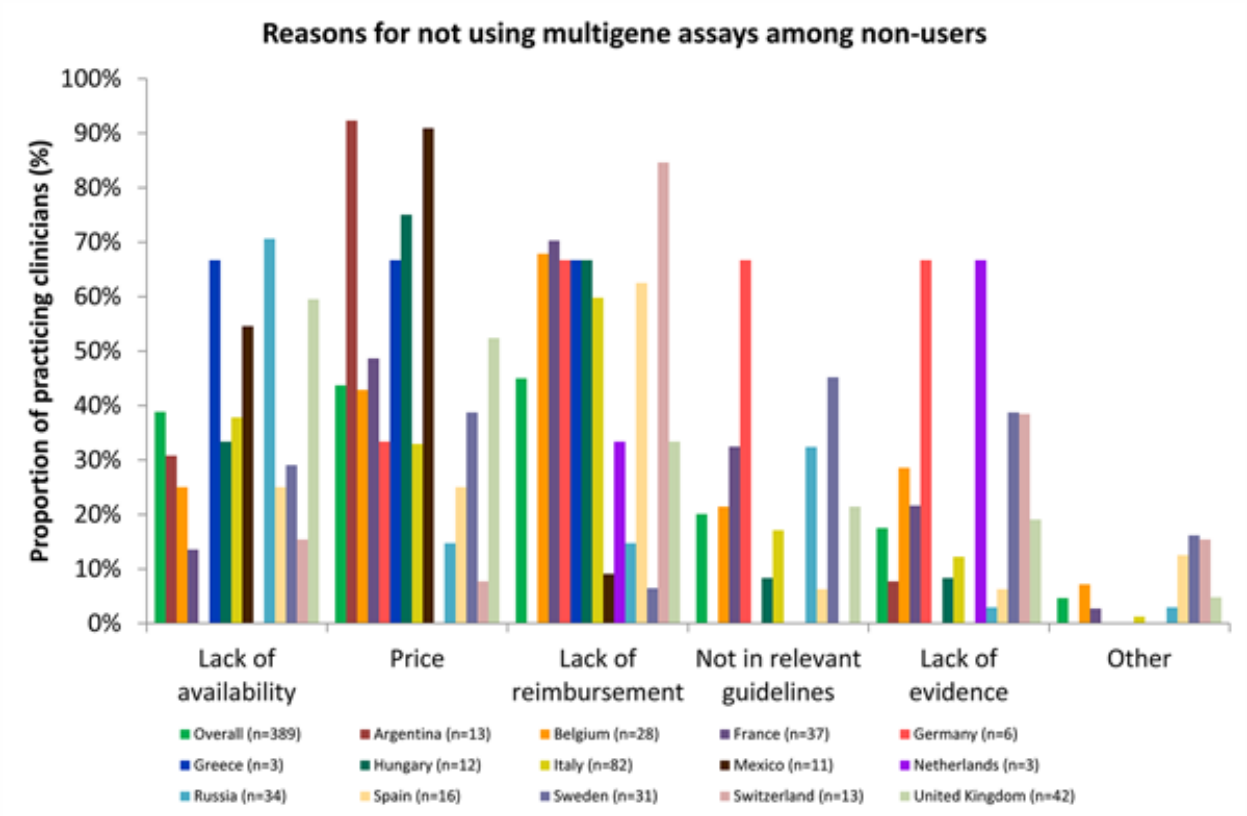
It has been demonstrated that multi-gene assays provide prognostic and predictive information beyond traditional parameters. Despite inclusion of some assays in the ESMO, ASCO, NCCN and St. Gallen guidelines for treatment of breast cancer, the access and use of multigene assays internationally is modest. The current use of different assays, the interest from physicians in using these assays, and the main reasons for not using the assays are poorly characterised.

The multidisciplinary application of genomics in clinical practise (MAGIC) survey aimed to identify criteria used to define the need for adjuvant chemotherapy for breast cancer and to characterise patients for whom the available data are sufficient for a decision and those for whom more data are required. The survey also assessed adoption of multi-gene assays, usage in practise, and reasons behind non-use.

The questions in the MAGIC survey questionnaire have been developed by international breast cancer experts. The SKIM group based in Rotterdam developed the Web online module.

From August 2013 to January 2014 an online survey was distributed among physicians with at least 5 years of experience in breast cancer treatment. Specific trends were evaluated; for smaller countries with populations less than 25 million inhabitants, it was required to obtain a minimum of 25 responses and for countries with more than 25 million inhabitants, at least 50 responses were needed.

Among eligible respondents, 643 physicians from 34 European countries completed the survey. Approximately 75% had more than 10 years of experience in diagnosis and treatment of breast cancer. Eleven countries had a sufficient number of responses to evaluate country specific trends (Belgium, Switzerland, France, Germany, Greece, Hungary, Italy, The Netherlands, Spain, Sweden, Switzerland, and UK).



Caption: MAGIC Survey results: Reasons for non-using multi-gene assays. © Matti Aapro

The results show that 51% of respondents use multi-gene assays in their clinical practise. A trend was observed that respondents who used multigene assays were more likely to request more information for a breast cancer patient profile than those who do not use multigene assays. The usage of multigene assays was higher among respondents who used tools or nomograms to estimate patient prognosis and respondents who did not consider Ki67% along with other existing pathology markers. Of those who had access to multigene assays, 38% indicated that they use them for more than 20% of their ER-positive, HER2-negative, node-negative patients and 22% used them for more than 20% of ER-positive, HER2-negative, node-positive patients.

Among respondents, there is a wide range of usage from less than 20% in Italy and Sweden to more than 80% in Germany, Greece and Netherlands.

The specific multi-gene assays used are Prosigna™ by 1% of respondents, FEMTELLE® by 2%, EndoPredict® by 5%, MammaPrint® by 15%, Oncotype DX® Breast Cancer Assay by 39% and other by 5%.

MammaPrint® is used most in The Netherlands and Spain. Oncotype DX is used most elsewhere. In Sweden no multi-gene assays are used.

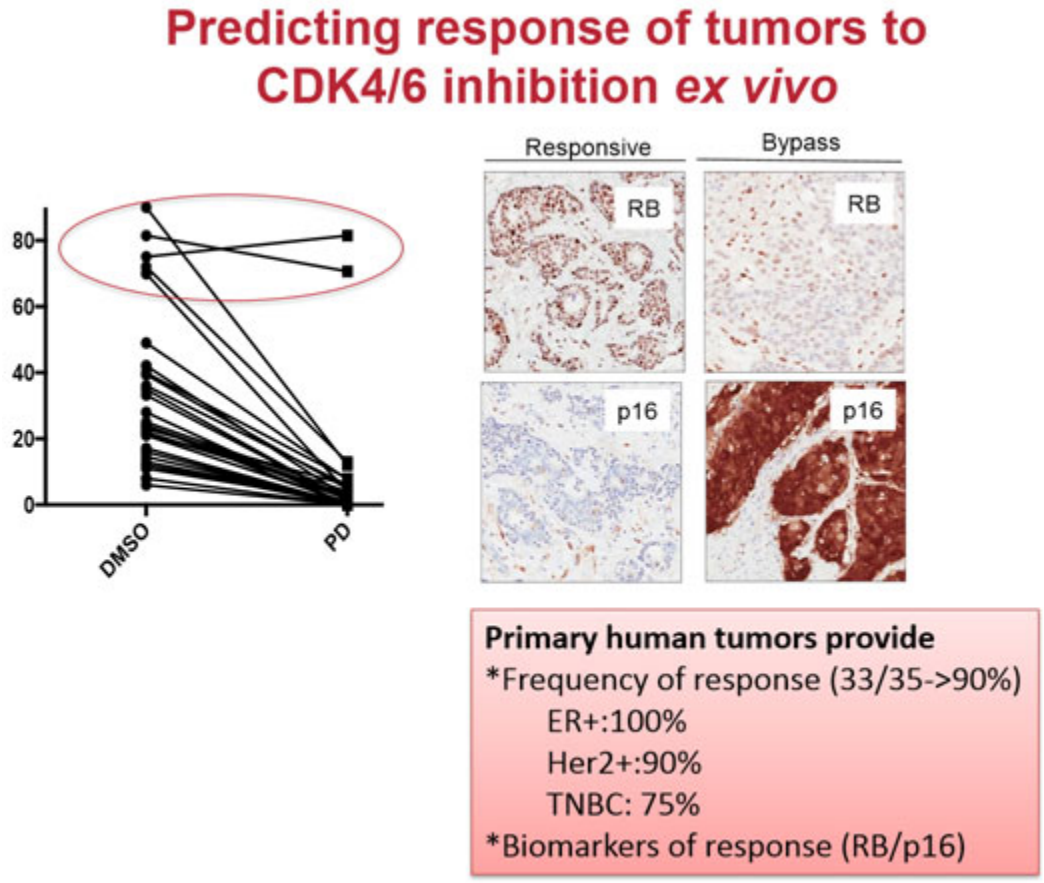
Of those who do not use multi-gene assays, 85% would like to incorporate them in their practise. They reported lack of reimbursement (51%), price (41%), no availability (35%), no recommendation in relevant guidelines (20%), and lack of evidence (19%) as reasons for no use.

Although the survey covered a small portion of breast cancer physicians in Europe, the findings reflects substantial heterogeneity in the adoption of multi-gene assays and in most cases under-usage with underlining problems in reimbursement, cost, and lack of availability, as main barriers for usage.

The survey was supported by an unrestricted grant from Genomic Health Inc.

Therapeutic effect and markers of response of palbociclib in HER2-positive breast cancer models

Cyclin-dependent kinases (CDK) 4 and 6 have been shown to be viable therapeutic targets in HER2-positive breast cancer models focused on pathways downstream of HER2. Here tissue based markers are defined to direct rational utilisation of the selective CDK4/6 inhibitor palbociclib (PD-0332991), using a combination of cell culture, mouse models, and human primary tumour explants. The results were presented by Dr Erik Knudsen of the Pathology Department, University of Texas Southwestern, Dallas in Best abstracts session.



Caption: Predicting response of tumours to CDK4/6 inhibition *ex vivo*. © Erik Knudsen

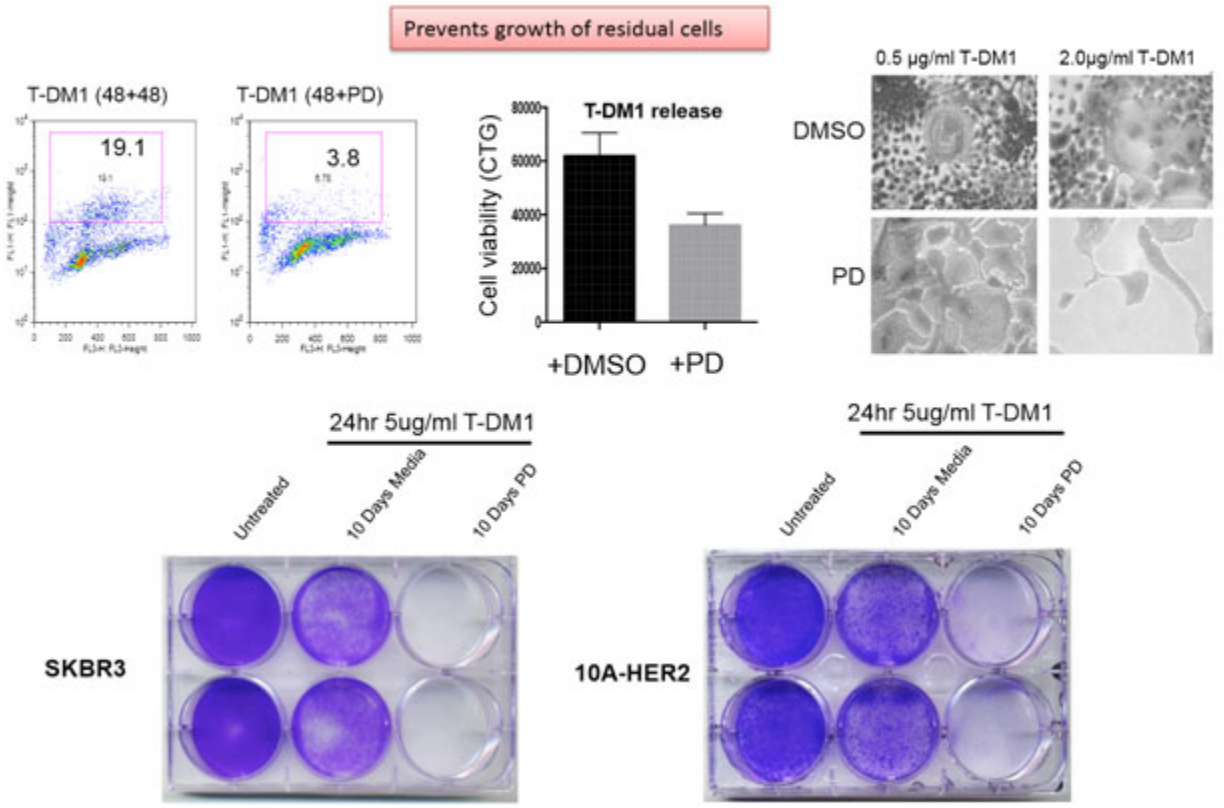
In spite of the efficacy of HER2 targeted therapies, recurrence and progression remain a challenge for treatment of advanced HER2-positive breast cancer. Mechanisms of bypass of HER2 targeted agents are complex. CDK4/6 controls a key pathway downstream of HER2. Inhibition of these kinases represents a therapeutic approach to augment the effectiveness of standard therapies.

Parallel studies evaluated the mechanisms of action in combination with the HER2-targeted agents lapatinib and trastuzumab emtansine (T-DM1).

CDK4/6 inhibition resulted in profound cytostatic arrest, induction of senescence, and inhibition of invasive properties in HER2-positive cell culture models. These data were recapitulated with significant suppression of Ki67 in the mouse model ($p < 0.05$) and HER2-positive xenografts ($p < 0.01$).

Furthermore, in a series of more than 20 primary breast tumour explants, treatment with palbociclib resulted in a greater than 5-fold suppression of the Ki67 ($p < 0.01$). These effects of palbociclib were dependent on an intact retinoblastoma (RB)-pathway. Loss of RB and high-levels of p16 were associated with resistance to CDK4/6 inhibition.

CDK4/6 inhibition can augment T-DM1 activity



Caption: CDK4/6 inhibition can augment T-DM1 activity. © Erik Knudsen

In models of acquired resistance to HER2-targeted therapies Cyclin D1 was inappropriately activated, and palbociclib treatment was effective at blocking proliferation by targeting this common pathway driving resistance.

Combination studies carried out in cell lines and primary tumour explants illustrated that palbociclib provides a complementary mechanism of action to T-DM1, and efficiently suppresses the proliferation of residual HER2-positive tumour cell populations that survive T-DM1.

The authors concluded that CDK4/6 inhibition has activity against HER2-positive cell culture models, xenografts, and tumour explants. Markers of resistance (p16 and RB) can be identified in clinical specimens. CDK4/6 inhibitor cooperates with multiple small molecules in HER2-positive models. It cooperates with T-DM1 to prevent growth of residual clones. Clinical studies of CDK4/6 inhibition in combination with HER2 targeted therapies have already commenced.

Dr Knudsen reported that he serves as an advisory board member and receives sponsored research funding from Pfizer. All other authors have declared no conflicts of interest.

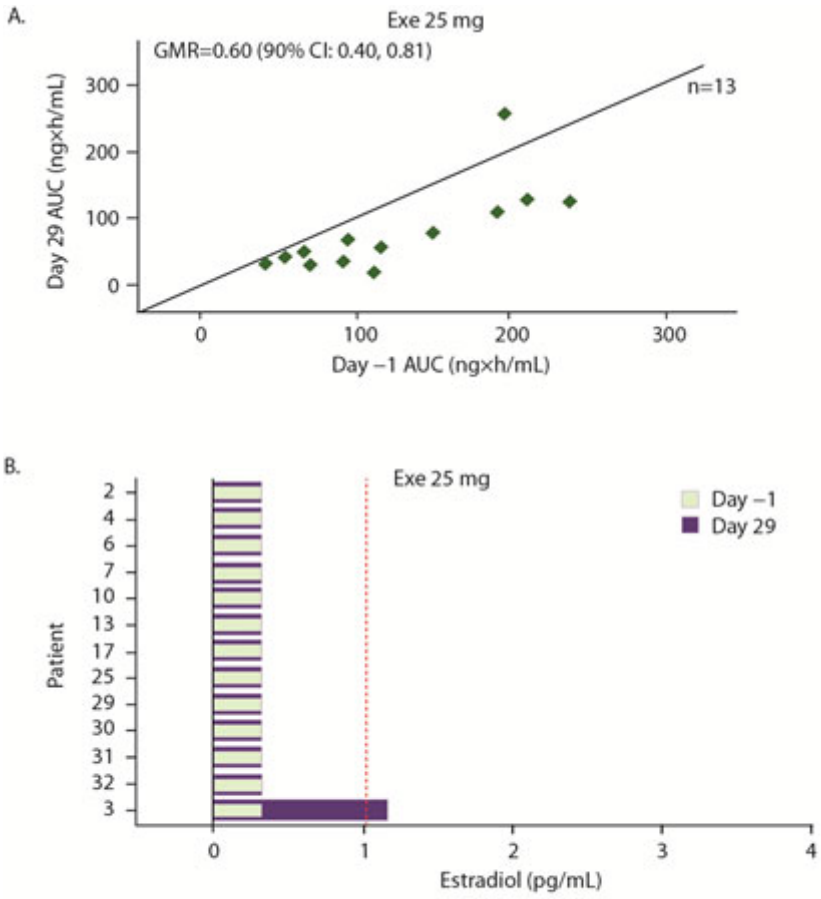
Dr Nicholas Turner, who discussed the study results, said that in a phase II trial of first-line treatment for ER-positive, HER2-negative advanced breast cancer, presented at AACR Annual

Meeting 2014, a combination of palbociclib and letrozole demonstrated a statistically significant improvement in progression-free survival. This phase II study proved the testing hypothesis that a combination of palbociclib and letrozole is better than letrozole alone in this patient subgroup.

A phase III study of palbociclib in combination with letrozole in same population of metastatic breast cancer is ongoing (PALOMA-2 study). A phase III study of palbociclib's combination with fulvestrant (PALOMA-3 trial) for metastatic breast cancers is also ongoing, as well as a combination with standard endocrine therapy (PENELOPE-B study) for certain early-stage breast cancers.

Enzalutamide with or without an aromatase inhibitor for advanced breast cancer

In a first phase I trial of enzalutamide alone or combined with an aromatase inhibitor in women with advanced breast cancer, pharmacokinetics and tolerability of enzalutamide were shown to be similar to that reported in men with metastatic castration-resistant prostate cancer. Enzalutamide reduced exposure to anastrozole by 88% and to exemestane by 40%. Oestradiol remained low in combination with exemestane and 12 of 36 patients enrolled into the aromatase inhibitor cohorts had been on study for 16 weeks or longer, according to the study results presented in a poster session by Dr Tiffany Traina of the Department of Medicine, Memorial Sloan-Kettering Cancer Center.



AUC=area under the curve; CI=confidence interval; exe=exemestane;
GMR=geometric mean ratio (AUC Day 29/AUC Day –1).
Dashed line represents expected estrogen levels with exemestane use.

Caption: Enzalutamide can be combined with exemestane and effective aromatase inhibition (as measured by serum plasma oestradiol levels) is maintained. © Tiffany Traina

Enzalutamide is a potent novel oral inhibitor of the androgen receptor which is expressed in a majority of breast cancers. It demonstrated preclinical activity in all breast cancer subtypes that express the androgen receptor and thus could be effective in androgen receptor-positive breast cancer, the authors explained in background of the study.

Androgens are converted by an enzyme, aromatase, to oestrone and oestradiol. Aromatase inhibitors block the conversion of androgens to oestrogens, resulting in an increase in androgens. Enzalutamide may add to the activity of aromatase inhibitors by blocking potential growth stimulation of the androgen receptor due to increased circulating androgens. Enzalutamide is a potent CYP3A4 inducer. Both exemestane and anastrozole are metabolised by CYP3A4. Stage 2 of this trial investigated whether any observed drug-drug interaction between enzalutamide and these aromatase inhibitors would translate into an effect on circulating oestrogens.

Stage 1 of this phase I study enrolled 15 patients with advanced breast cancer evaluated single agent enzalutamide at daily doses of either 80 or 160 mg. Blood was collected for

pharmacokinetic analysis. Dose limiting toxicities were recorded through day 35. This stage included patients confirmed 160 mg of enzalutamide as the daily dose for further study in women with advanced breast cancer. A single dose limiting toxicity, in particular adrenal insufficiency, occurred at dose of 80 mg.

Stage 2 of the trial evaluated daily enzalutamide at 160 mg dose and added to patients receiving either anastrozole 1 mg or exemestane 25 mg. In stage 2, blood for pharmacokinetics and hormones was collected pre- and post-enzalutamide treatment.

Tumour assessments were performed approximately every 3 months in both stages.

As of October 2013, 20 patients were enrolled to receive anastrozole and 16 to receive exemestane. Median age was 57 years, performance status at baseline was ECOG PS 1, and patients received an average of 5 prior therapies for advanced disease in stage 1 study. However, in stage 2 the median age was 59 years, performance status was 0 and the patients received an average of4 prior lines of therapy for their cancer.

In stage 1 of the study, common (>15%) treatment-related adverse events of any grade included AST elevation, nausea, and nasal congestion. In stage 2 common treatment-related adverse events of any grade were fatigue, anorexia, nausea, hot flush, and vomiting. Grade ≥3 adverse events in at least 2 patients were anaemia in stage 1 and fatigue in stage 2.

The geometric mean ratio of day 29/day 1 AUC for anastrozole was 0.12 and for exemestane 0.60, meaning that enzalutamide reduced exposure to anastrozole by 88% and to exemestane by 40%.

Preliminary hormone data showed increased oestradiol on day 29 over day 1 in 7 of 14 patients in anastrozole group vs. 1 of 12 in exemestane group. The authors concluded that oestradiol remained low in combination with exemestane, but possibly not with anastrozole.

Exemestane with or without enzalutamide is being evaluated in randomised phase II studies. Three global phase II clinical trials are enrolling:

- MDV3100-11: single agent enzalutamide in androgen receptor positive triple negative breast cancer with primary endpoint of clinical benefit rate.
- MDV3100-12: a randomised trial investigating exemestane plus enzalutamide vs. exemestane plus placebo in hormone receptor positive breast cancer with a primary endpoint of progression-free survival.
- 9785-CL-1121: an open label study investigating enzalutamide with trastuzumab in HER2-positive, androgen receptor positive metastatic or locally-advanced breast cancer with a primary endpoint of clinical benefit rate of at least 24 weeks.

Dr Traina serves as an advisory board member for Genentech, Eisai and Prostrakan. She received honoraria from Genentech, Celgene, Merck, Eisai and Prostrakan. She received research funding from Medivation, AstraZeneca, Eisai, Ziopharm, Janssen, Genentech and Novartis. Other authors disclosed that Dr Yardley provides consultancy and is advisory board member for Medivation; Dr Patel receives honoraria for a speakers bureau from Medivation; Dr Blaney is an employee of Medivation; Dr Gibbons is an employee of Medivation; and Dr

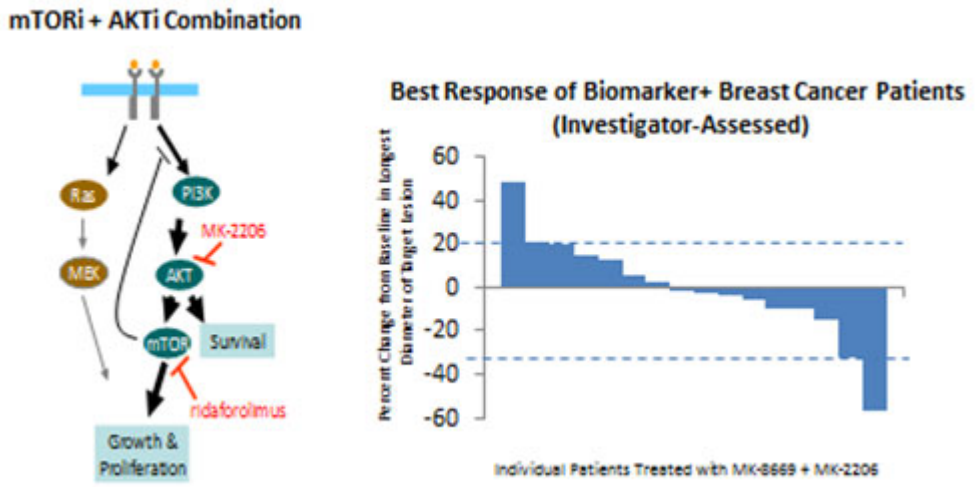
LoRusso receives research grant and provides consultancy for Astellas. All other authors have declared no conflicts of interest.

Combination of mTOR and AKT inhibitors in patients with advanced breast cancer

A combination of mTOR inhibitor, ridaforolimus and novel AKT inhibitor, MK-2206 showed activity in heavily pretreated hormone positive and negative breast cancer patients who exhibit PI3K pathway dependence based on low RAS signature score. The combination was well tolerated in a phase I study. The results provide a rationale for exploring this combination in further studies, according to Dr Shilpa Gupta of the Lee Moffitt Cancer Center who presented the study in a poster session on new drug development.

The PI3K/AKT/mTOR signalling pathway is aberrantly activated in various cancers, including breast cancer. The study rationale was that a combination of mTOR inhibitor and AKT inhibitor can lead to complete blockade of this pathway and disrupt tumour cell proliferation, metabolism and survival signalling.

In a phase I study, ridaforolimus and MK-2206 were tested in advanced cancers. The study included a dose expansion cohort of enriched breast cancer patients with low RAS gene signature in archival or fresh tissue. RAS signature was a RNA transcription assay that derived a score from the expression of 147 transcripts. In order to be eligible for the study, patients with ER-positive breast cancer had also to demonstrate a high Ki67 index.



Caption: Novel combination of MK-8669 (Ridaforolimus) + MK-2206 (AKT inhibitor) in patients with advanced breast cancer with PI3K pathway dependence. © Gupta Shilpa

In total, 124 heavily pretreated breast cancer patients were pre-screened for the study with 52% being biomarker-eligible. The total number of patients with breast cancer was 17.

Maximum-tolerated dose for ridaforolimus was 10 mg per day during 5 days in a week plus 90 mg of MK-2206 as a weekly dose. One of 17 patients experienced a dose limiting toxicity of grade 3 rash.

Median treatment duration was 2 cycles. Complete response (CR) was defined as disappearance of non-nodal target lesions or reduction of nodal lesions to less than 10 mm in short axis. Partial response (PR) was defined as at least 30% decrease from baseline in sum of diameters/volumes of target lesions. Objective responses as assessed by investigators by RECIST 1.1 criteria were seen in 2 out of 16 patients (12,5%), both patients experienced PR . By volumetric 3D tumour assessment, objective responses were seen in 4 out of 14 patients (28,6%), with 2 PR and 2 CR. Stable disease of at least 6 months was seen in 1 patient.

The combination was well tolerated and main adverse events were rash (44.4%), stomatitis (38.9 %), diarrhoea (27.8%), anorexia (27.8%) and fatigue (22.2%).

The authors concluded that the combination treatment resulted in disease responses in some heavily pretreated patients. The treatment was generally well tolerated with rash, asthenia, diarrhoea and stomatitis as most common drug-related adverse events and with less than 6% of patients experiencing a grade 3 event.

Among study authors J. Cheng, R. Wang, A. Swift and A. Tosolini are scientists in Merck Labs. All other authors have declared no conflicts of interest.

Related Information

Click Here to access the Conference abstracts.

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IMPAKT Breast Cancer Conference 7-9 May 2015, with the pre-conference training course, 6-7 May 2015.

Affiliation and Disclosure

Affiliation

Dr Svetlana Jezdic, ESMO Head Office.

Disclosure

No conflicts of interest to disclose.

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